

KOUZARIDES

Appl. No. 09/744,469

September 22, 2003

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Withdrawn) An assay method for an agent which affects Importin α acetylation, the method including:

(a) treating an acetylated Importin α polypeptide or peptide with a test compound, or

(b) treating with a test compound an Importin α polypeptide or peptide which comprises one or more lysine residues corresponding to those found at positions Lys 18, Lys 20 and Lys 22 in wild-type Importin α Rch1, in which polypeptide or peptide one or more of said lysines is not acetylated, or

(c) bringing into acontact a substance which includes a CBP polypeptide which acetylates Importin α , a substance which includes an Importin α polypeptide or peptide including a site acetylated by CBP, and a test compound; and

(d) determining acetylation of the Importin α polypeptide or peptide.

2. (Currently Amended) An assay method for identifying an agent which affects one or more Importin α activities selected from the group consisting of (i) binding Importin β , (ii) translocating into the nucleus, and (iii) importing a cargo protein; , the method ~~including~~ comprising:

(a) ~~bringing into contact~~ contacting Importin α and a test compound;

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(b) determining said one or more Importin α activities in the presence and absence of a CBP (~~CREB binding protein~~) (cAMP-response element binding protein) polypeptide which acetylates Importin α , thereby determining the effect of said polypeptide CBP on said one or more activities; and;

(c) comparing said effect in the presence of ~~test compound with said effect in~~ and absence of the test compound, wherein a difference in said effect in the presence relative to the absence of the test compound being indicative that said test compound is an agent which affects said one or more Importin α activities.

3. (Cancelled).

4. (Currently Amended) An assay method for identifying an agent which ~~modulates interaction between~~ inhibitory modulator of CBP (cAMP-response element binding protein) and Importin α binding, the method ~~including~~ comprising:

(a) ~~bringing into contact a first substance including~~ contacting a CBP polypeptide, ~~or peptide, a second substance including an Importin α polypeptide or peptide, and a test compound under conditions in which, in the absence of the test compound being an inhibitor, the first and second substances interact;~~

(b) ~~determining interaction between the first and second substances,~~ the CBP polypeptide and Importin α polypeptide binding by comparison to CBP polypeptide and Importin α polypeptide binding in the absence said test compound; and;

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~~(c) comparing said interaction in the presence of test compound with said interaction in the absence of test compound;~~

~~a difference said interaction in the presence relative to the absence of test compound being indicative that said test compound is an agent which modulates interaction between CBP and Importin α wherein inhibition of CBP polypeptide and Importin α polypeptide binding identifies said test compound as an inhibitory modulator of CBP and Importin α binding~~

5. (Currently Amended) An assay method for identifying an agent which affects one or more activities of Importin α selected from the group consisting of (i) binding Importin β , (ii) translocating into the nucleus, and (iii) importing a cargo protein the method comprising:

(a) ~~bringing into contact~~ contacting CBP (cAMP-response element binding protein) and a test compound;

(b) determining CBP acetyltransferase activity in the presence and absence of a test compound; and

(c) comparing said CBP acetyltransferase activity in the presence ~~of test compound with said activity in the~~ and absence of test compound, wherein CBP acetylates Importin α and wherein a decrease in CBP acetyltransferase activity in the presence relative to the absence of the compound being indicative that the test compound is an agent which affects said one or more activities of Importin α ; and

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(d) determining the effect of the test compound on one or more activities of Importin α selected from the group consisting of (i) binding Importin β , (ii) translocating into the nucleus and (iii) importing a cargo protein.

6. (Currently amended) A The method according to claim 5 comprising determining acetylation of Importin α by CBP.

7 and 8 (Cancel).

9. (Currently Amended) An assay method for identifying an agent which ~~interacts with~~ binds to a region of CBP (cAMP-response element binding protein) or a region of Importin α ,

~~which wherein the region of CBP interacts with Importin α and binds to the region of Importin α where acetylation of Importin α occurs which region of Importin α interacts with CBP, a said~~, an agent which interacts with binds to said region of CBP or said region of Importin α being ~~a candidate~~ an inhibitory modulator of interaction between CBP and Importin α binding, the method ~~including~~ comprising:

(a) ~~bringing into contact a substance which includes~~ contacting CBP or a CBP peptide comprising residues 1078 to 1785 of CBP which interacts with Importin α , or which includes an Importin α or an Importin α peptide comprising residues 1 to 95 of Importin α peptide which interacts with CBP, and a test compound; and

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(b) determining ~~interaction~~ the binding between ~~said substance~~ the CBP, CBP peptide, Importin α or Importin α peptide and the test compound,

~~the presence of interaction between said substance and the test compound being~~
~~indicative that the test compound is a said agent~~

wherein inhibition of CBP or CBP peptide and Importin α or Importin α peptide binding identifies said test compound as an inhibitory modulator of CBP and Importin α binding.

10. (Thrice amended) A The method according to claim ~~1~~ 2, claim 4, claim 5 or claim 9 further comprising formulating a said agent into a composition comprising a pharmaceutically acceptable excipient.

11. (Cancelled).

12. (Withdrawn) A method according to claim 10 further comprising providing a said agent, or, optionally where said agent is peptidyl, nucleic acid encoding a said agent, to cells to modulate one or more of ability of Importin α to (i) bind Importin β , (ii) translocate into the nucleus, and/or (iii) import a cargo protein.

13. (Withdrawn) A method according to claim 12 wherein said agent or nucleic acid is provided to cells *in vitro*.

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14. (Withdrawn) A method according to claim 10 further comprising use of a said agent, or, optionally where said agent is peptidyl, nucleic acid encoding a said agent, in the manufacture of a medicament for treating a disorder of Importin α activity.

15. (Withdrawn) A peptide fragment of Importin α or of CBP, which peptide is about 40 amino acids or less, and which modulates interaction between Importin α and CBP.

16. (Withdrawn) A peptide according to claim 15 which is an Importin α peptide comprising one or more lysine residues corresponding to those found at positions Lys 18, Lys 20 and Lys 22 in wild-type Importin α .

17. (Withdrawn) A peptide according to claim 16 which is about 20 amino acids in length.

18. (Withdrawn) An isolated nucleic acid encoding a peptide according to claim 17.

19-23 (Cancelled).